Thus under conditions of hunger there is a bimodal distribution of interspike intervals, which changes under the influence of distension of the stomach and becomes monomodal in character. Investigations on frogs essentially confirmed the results of experiments on other models [5] and showed that specific motivational excitation not only has a characteristic pattern of stochastic structure at the center, but it also spreads out to peripheral nerve formations.

We consider that this observation provides a deeper insight into the physiological mechanisms of functional mobility, discovered by Snyakin, and it suggests that the physiological nucleus realizing the above mechanisms is a motivating mechanism, and that the method of coding of the motivating excitation rests on a holographic basis.

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ABOLITION OF THE INHIBITORY EFFECT OF ANTIBODIES TO S-100 PROTEINS ON THE CALCIUM CURRENT OF SNAIL NEURONS BY INTRACELLULAR INJECTION OF EGTA

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Antibodies to the S-100 fraction of bovine brain proteins depolarize the membrane and inhibit action potential generation in neurons of Helix pomatia [5], and under voltage clamp conditions they inhibit the total inward current through voltage-dependent channels [7]. The effect of antibodies to these proteins on voltage-dependent Ca conductance has not been studied. Meanwhile the ability of these proteins to bind Ca^{++} ions [8, 12] suggests that they play a role in the regulation of conductance of Ca channels of the neuronal membrane.

Accordingly, it was decided to study the effect of antibodies to S-100 proteins on the voltage-dependent Ca current (I_{Ca}) of snail neurons and to elucidate the mechanisms of these effects.

EXPERIMENTAL METHODS

Unidentified neurons of Helix pomatia were isolated by means of fine needles without preliminary treatment of the preparation with proteolytic enzymes, and placed in a continuously flowing solution of the following composition (in mM): $CaCl_2$ – 10; KCl – 4; $MgCl_2$ – 4, tetraethylammonium bromide – 95; 4-aminopyridine – 5; Tris-HCl – 5 (pH 7.6). If the volume

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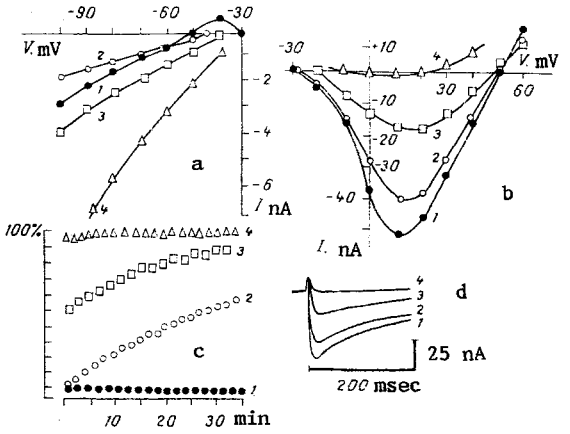

Fig. 1. Inhibition of I_{Ca} of a single neuron after application of various concentrations of AS-100. 1) Measurements of transmembrane current in control solution; 2, 3, 4) the same measurements after addition of 2, 4, and 6 μg/ml of AS-100 respectively to external solution. a, b) Current-voltage characteristics of membrane plotted for shift of membrane potential from −60 mV by stimuli 40 msec in duration, of different sign and amplitude, relative to values of current at end of stimuli. Measurements made 10 min after application of AS-100; c) inhibitory effect of AS-100 on peak amplitude of maximal I_{Ca} as a function of time. Ordinate) inhibition of I_{Ca} (in %); d) trace of I_{Ca} at +10 mV 10 min after application of AS-100.

of solution in the chamber was not less than 2 ml, when the flow was stopped the isolated neurons could generate an I_{Ca} of stable amplitude for several hours. The voltage was recorded and substances injected intracellularly through a three-barreled microelectrode. The barrel for recording voltage and the reference barrel for microionotophoresis were filled with 2 M K^+ citrate solution. Their resistance was 5-10 MΩ. The barrel for injection was filled with 0.2 M EGTA solution (Serva, West Germany). The pH of the EGTA solution was adjusted to 7.6 with crystalline KOH. The strength of the injection current was 10 nA. Between injections, a blocking current of 2 nA was used. To pass the current for voltage clamping a separate single-barreled microelectrode with a resistance of 5-10 mΩ was inserted into the neuron. The SFZ-1100, MEZ-7107 electronic system (Nihon Kohden, Japan) was used for voltage clamping and recording ionic currents. The holding potential was set at −60 mV. During stepwise depolarization with a duration of 40-200 msec an I_{Ca} could be recorded with a maximum at between 0 and +20 mV. The voltage and current were recorded on an RJG-4002 automatic ink-writer ("Nihon Kohden").

Rabbit antibodies to the S-100 fraction of bovine brain proteins (AS-100) were isolated by the immunoaffinity method [1] at the P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR. Before use in the experiments the antibodies were kept in a frozen solution of 0.15 M NaCl, with a protein concentration of 1 mg/ml. The gamma-globulin fraction of serum proteins of an unimmunized rabbit, which was used in the control experiments, was kept under the same conditions. Proteins were added by means of a microsyringe into the working chamber when the flow was interrupted.

EXPERIMENTAL RESULTS

Blood serum proteins of the gamma-globulin fraction of unimmunized rabbits, in a concentration of 2-10 μg/ml, had no marked effect on the steady-state current at the holding potential, on the amplitude of I_{Ca}, or on the position of the maximum of I_{Ca} along the voltage axis (n = 8).

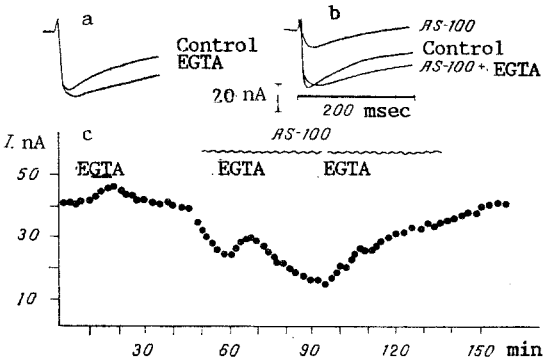

Fig. 2. Abolition of inhibitory effect of AS-100 on I_{Ca} by intracellular injection of EGTA. a, b) Traces of maximal I_{Ca} before injection and after injection of EGTA in control solution (a) and in presence of 2 μg/ml of AS-100 (b); c) peak amplitude of I_{Ca} before and after application of 2 μg/ml of AS-100 (wavy line). Injections of EGTA indicated by straight lines.

Application of AS-100 in a concentration of 2-6 μg/ml induced an inward current at the holding potential level and a decrease in I_{Ca} or even its total inhibition (n = 15). The effects of AS-100 on transmembrane currents were virtually completely reversible when the neuron was rinsed with the control solution for 10-30 min, so that the action of various concentrations of AS-100 could be studied on the same neuron. A typical example of the change in transmembrane currents of a nerve cell in the presence of different concentrations of AS-100 is given in Fig. 1. The current-voltage characteristic (CVC) of the membrane in the region of negative potentials, below the threshold for opening Ca channels, shows (Fig. 1a) that, depending on the AS-100 concentration, the steady-state inward current may be linked with a decrease in membrane conductance (curve 2), with the absence of any change in total conductance (curve 3), or with an increase in conductance (curve 4). However, irrespective of the character of the change in ohmic conductance of the membrane, I_{Ca} was reduced by different concentrations of AS-100. The effect of AS-100 on I_{Ca} was dose-dependent (Fig. 1b, d) and depended on the incubation time (Fig. 1c). No significant shift of the maximum of CVC of I_{Ca} along the voltage axis was observed in most experiments (Fig. 1b). In some experiments application of AS-100 not only lowered the peak amplitudes of I_{Ca}, but also led to a marked increase in I_{Ca} decay and to the appearance of an outward current. In a concentration of over 6 μg/ml, AS-100 usually caused irreversible damage to the neuron.

Inhibition of I_{Ca} in the present experiments could evidently be caused both by the direct action of antibodies on Ca channels and by their indirect effect on I_{Ca} through accumulation of free intracellular Ca [4, 9, 10]. To solve this problem, effects of intracellular injection of the Ca-binding agent EGTA on I_{Ca} in control solution and after application of AS-100 were studied on five cells. The results of one of these experiments are given in Fig. 2. After injection of EGTA into a neuron placed in the control solution the peak amplitude of I_{Ca} was increased, and its decay was delayed (Fig. 2a). Against the background of the action of AS-100, when I_{Ca} was largely inhibited, injection of EGTA caused partial or total recovery of I_{Ca} (Fig. 2b). Similar results were obtained in the remaining four experiments.

Abolition of the inhibitory effect of AS-100 on I_{Ca} after intracellular injection of EGTA is evidence that AS-100 has no direct action on Ca channels. The mechanism of the observed inhibition of I_{Ca} is evidently an increase in the free Ca concentration in the cytoplasm and Ca-dependent inactivation of Ca channels [4, 9, 10].

The causes of accumulation of free Ca in the cytoplasm under the influence of AS-100 are not quite clear. After application of high concentrations of the antibodies, inducing an increase in ohmic conductance of the membrane and a steady inward current, entry of Ca^{++} ions into the cell from outside is perfectly expectable. However, lower concentrations of antibodies do not cause an increase in steady-state membrane conductance. In this case, one can only assume the release of bound intracellular Ca and (or) inhibition of systems pumping Ca^{++} from the cytoplasm [2, 4]. When release of bound intracellular Ca^{++} is mentioned, the possibility of weakening of binding of Ca^{++} ions by S-100 proteins on interaction with antibodies is mainly implied.

The mechanism of inhibition of I_{Ca} under the influence of S-100 described in this paper is not universal and does not extend to effects of antibodies to other proteins. For example, antibodies to the microsomal fraction of rat brain not only do not inhibit but, on the contrary, they stimulate I_{Ca} of snail neurons [6]. Meanwhile antibodies to antigens of snail nerve tissue, just as in the present investigation, caused a decrease in the amplitude of I_{Ca} [3]. However, the authors cited do not connect these changes with the accumulation of free intracellular Ca and they explain them by the direct action of antibodies on ionic channels. Other investigators, who observed inhibition of I_{Ca} of Paramecium under the influence of antibodies to antigens of the cilia of these cells, reached the same conclusion because this effect was not abolished by intracellular injection of EGTA [11].

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